Coccidioidomycosis: Early Immunologic Findings

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T and B lymphocyte number and lymphocyte response to phytohemagglutinin, pokeweed mitogen, concanavalin A, coccidioidin and streptokinase-streptodornase (SKSD), plus monocyte ingestion of coccidioidin- and IgG-coated chicken erythrocytes were measured in 5 patients with coccidioidal meningitis, 11 with nonmeningeal extrapulmonary coccidioidomycosis and 5 with localized pulmonary infections. These cases were evaluated within six months of the onset of infection. Lymphocytic responses to phytohemagglutinin, pokeweed mitogen, concanavalin A, SKSD and coccidioidin and monocytic ingestion of coccidioidin- and IgG-coated chicken erythrocytes were severely decreased in patients with meningeal and nonmeningeal, extrapulmonary coccidioidomycosis but not in patients with localized pulmonary infections. T and B cell numbers, however, were normal in all groups. Thus, defects in cellular immunity are involved in the pathogenesis of extrapulmonary coccidioidomycosis and measurements of lymphocytic and monocytic function may identify patients prone to extrapulmonary infection.

The cellular immune response is a critical factor in the pathogenesis of coccidioidomycosis.[1,2] Several studies have shown that impairments in skin hypersensitivity and lymphocytic response to coccidioidin are common in patients with disseminated disease but not in those with less severe pulmonary infections.[3-5] Because patients in these studies had their infection for extended periods of time, information on the immune response in the early stages of coccidioidomycosis is limited. A unique opportunity to study this relationship occurred in 1978 when an epidemic of coccidioidomycosis followed a severe dust storm in the Sacramento Valley.[6] This report describes lymphocyte and monocyte function in 5 patients with coccidioidal meningitis, 11 with nonmeningeal extrapulmonary coccidioidomycosis and 5 with localized pulmonary infection, all of whom were infected for less than six months.

Methods

Patients

Clinical data for the patients in whom coccidioidomycosis developed following the dust storm of December 20 and 21, 1977, are summarized in Table 1. The five patients with meningitis had a positive culture of a biopsy specimen from a meningeal or nonmeningeal site and complement-fixing antibody in their cerebrospinal fluid. Cases diagnosed as disseminated nonmeningeal coccidioidomycosis had *Coccidioides immitis* in biopsy specimens or cultures from extrapulmonary sites. These patients were treated with amphotericin B or amphotericin B methyl ester. Patients with pulmonary coccidioidomycosis had roentgenographic evidence of infection and serum complement-fixing antibody. The pulmonary infection was self-limiting in four cases and persistent in one (No. 21).

Immunologic Studies

Immunologic tests for delayed hypersensitivity to coccidioidin, T and B lymphocyte and monocyte function and complement-fixing antibody to *C immitis* were carried out from two to six months following the onset of illness. Skin tests were done by intradermal inoculation of 0.1 ml of coccidioidin (Cutter Laboratories,

Refer to: Miller CH, MacKenzie MR, Paglieroni T, et al: Coccidioidomycosis: Early immunologic findings. West J Med 1983 Jan; 138:55-59.

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This study was supported by Public Health research grant 17192-06 from the National Institutes of Health and grant ES-01327 from the National Institute of Environmental Health Sciences.

Submitted, revised, July 21, 1982.

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ABBREVIATIONS USED IN TEXT

E=sheep erythrocytes
SKSD=streptokinase-streptodornase

1:100). Indurations that were 0.5 cm or greater at 48 hours were recorded as positive.

Surface Markers

Venous blood was collected in syringes containing 20 units of preservative-free heparin per milliliter of blood. Monocytes were removed from these preparations by adherence to glass surfaces or by the ingestion of carbonyl iron.[7] The remaining mononuclear cells were separated on Ficoll-sodium metrizoate (Hypaque) gradients.[8] The number of sheep erythrocyte receptor-bearing cells (E), complement receptor-bearing cells and surface immunoglobulin-bearing cells was determined on monocyte-depleted cell populations by methods previously described.[9]

Lymphocyte Transformation Assays

Lymphocyte transformation was tested by incubation with mitogens and antigens. Gradient-isolated mononuclear cells (phagocytic cells were not removed) in RPMI 1640 were stimulated with 25 μg per culture of phytohemagglutinin and concanavalin A, 5 μl per culture of pokeweed mitogen, 50 μl of coccidioidin dilutions that ranged from 1:10 through 1:512 and 50 units per ml of streptokinase-streptodornase (SKSD). The coccidioidin was dialyzed extensively against saline before use. The SKSD was reconstituted from the lyophilized product in 0.01 mol per liter phosphate-buffered saline at a pH of 7.5 and stored at a concentration of 1,000 units per ml.[9]

Monocyte Function

The ability of freshly collected monocytes to ingest chicken erythrocytes that were coated with coccidioidin or human gamma globulin (IgG) was determined by the method of Huber and Fudenberg.[10] Antigen coating was achieved by incubating 10 ml of a washed 2 percent suspension of chicken erythrocytes for ten minutes with an equal volume of 0.05 mg per ml of tannic acid solution. Ten million tannic acid-treated chicken erythrocytes were reacted with 1.0 mg per ml of IgG, or 0.1 ml of the dialyzed coccidioidin solution. The appropriate coccidioidin dilution was determined by hemagglutination inhibition testing.[11] The antigen-coated chicken erythrocytes were washed in phosphate-buffered saline, centrifuged at 1,500 rpm and resuspended in 1 ml of phosphate-buffered saline. The presence of antigen on the erythrocyte surface was confirmed by hemagglutination testing. A suspension containing antigen-coated or control chicken erythrocytes in RPMI 1640 containing 10 percent fetal calf serum was added to 5×10^4 monocytes that had previously settled onto tissue culture chamber slides (Lab-Tek). The mixture was incubated for 60 minutes. After washing, the cells were fixed in 100 percent methanol and stained with a Wright's-Giemsa mixture; 200 monocytes were counted and the percentage of cells with two or more ingested chicken erythrocytes was recorded.

Results

Table 2 shows lymphocyte surface marker patterns in patients with coccidioidomycosis and in normal persons. The surface marker patterns of lymphocytes from patients with meningeal, nonmeningeal, disseminated and pulmonary infections were similar to those observed in persons whose skin test results were negative,

TABLE 1.—*Clinical Characteristics of Patients in Whom Coccidioidomycosis Developed Following the Dust Storm of December 20 and 21, 1977*

Case No.	Age	Sex	Race	Site of Infection	Skin Test Result	Complement Fixation Test	Response
1	26	♂	White	Meningeal	Neg	1:32	Died
2	50	♂	White	Meningeal	Neg	1:256	Died
3	53	♂	Black	Meningeal	Neg	1:64	Died
4	60	♂	White	Meningeal	Pos	1:32	Recovered
5	66	♂	Black	Meningeal	Neg	1:32	Died
6	21	♂	Black	Disseminated	Pos	1:256	Died
7	26	♂	Black	Disseminated	Pos	1:16	Recovered
8	29	♀	Black	Disseminated	...	1:256	Recovered
9	34	♂	Black	Disseminated	Neg	1:128	Recovered
10	36	♂	Black	Disseminated	Neg	1:256	Recovered
11	41	♂	Black	Disseminated	Neg	1:32	Died
12	43	♂	Black	Disseminated	...	1:16	Recovered
13	46	♂	Mex-Amer	Disseminated	Neg	1:16	Recovered
14	48	♂	Black	Disseminated	...	1:64	Recurrence
15	61	♂	Black	Disseminated	...	1:64	Died
16	77	♂	White	Disseminated	Pos	1:2	Recovered
17	24	♀	White	Pulmonary	Pos	1:4	Recovered
18	27	♂	Black	Pulmonary	Pos	1:64	Recovered
19	32	♀	Mex-Amer	Pulmonary	Pos	1:128	Recovered
20	42	♂	White	Pulmonary	Pos	1:256	Recovered
21	50	♀	Mex-Amer	Pulmonary	Pos	1:16	Persistence

except for the E rosette marker, which was significantly lower in disseminated disease.

Lymphocyte transformation responses following stimulation with mitogens and antigens are shown in Figures 1 and 2. Patients with meningeal and nonmeningeal disseminated coccidioidomycosis had similar and significantly reduced ($P<.05$) responses to phytohemagglutinin, pokeweed mitogen and concanavalin A when compared with responses of cells from normal persons or patients with pulmonary coccidioidomycosis (using Student's t test). Lymphocyte transformation was affected in the same manner in the studies that tested responses to coccidioidin and SKSD. That is, the lymphocytes of patients with meningeal and disseminated nonmeningeal coccidioidomycosis did not respond to coccidioidin and responded less well to SKSD than did the lymphocytes of patients with pulmonary infection, which showed fivefold increases in blastogenic response to coccidioidin ($P<.01$) and normal blastogenic responses to SKSD. No blastogenic response to coccidioidin occurred in patients with dermal hypersensitivity to coccidioidin or in those with anergy. No significant difference between patient groups and normal persons was noted in the unstimulated cultures.

TABLE 2.—*Lymphocyte Surface Markers in Patients With Coccidioidomycosis*

Diagnosis	Number of Patients	E Rosette Mean %	E Rosette SD	EAC Rosette Mean %	EAC Rosette SD	SIg Mean %	SIg SD
Normal	20	63	±8	16	±5	20	±5
Meningitis	5	50	±17	23	±10	31	±17
Disseminated	11	42	±13*	27	±12	31	±12
Pulmonary	5	61	±7	17	±7	17	±5

Abbreviations: E = sheep erythrocyte receptor-bearing cells, EAC = complement receptor-bearing cells, SIg = surface immunoglobulin-bearing cells, SD = standard deviation.

*$P<.01$ when compared with normal values.

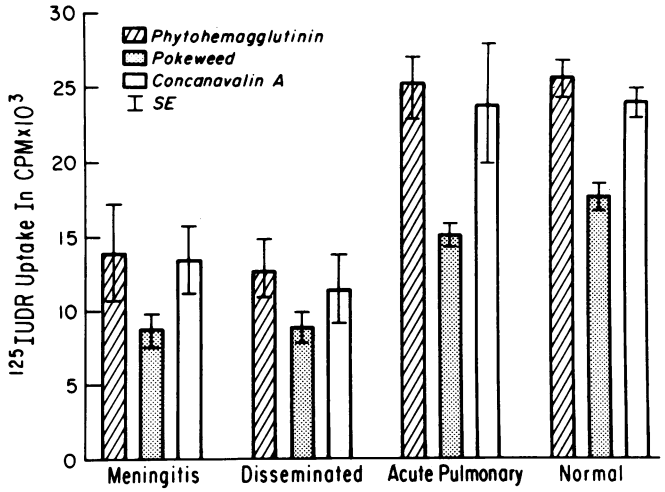

Figure 1.—Lymphocyte responses to mitogens in patients with different forms of coccidioidomycosis. CPM = counts per minute, ^{125}IUDR = iododeoxyuridine I 125, SE = standard error.

Findings from the experiments testing the ability of monocytes from patients with different forms of coccidioidomycosis to ingest coccidioidin- or IgG-coated chicken erythrocytes are depicted in Figures 3 and 4. In general, monocytes from patients with pulmonary coccidioidomycosis showed enhanced ingestive capacities for coccidioidin-coated chicken erythrocytes when compared with monocytes from patients with meningeal or disseminated nonmeningeal infections. Monocytes from the latter groups ingested coccidioidin-coated chicken erythrocytes at the same rate as that observed in monocytes from normal, presumably uninfected controls. The same pattern was observed when human IgG was used as the antigen, except that in these experiments monocytes from normal persons had the same high

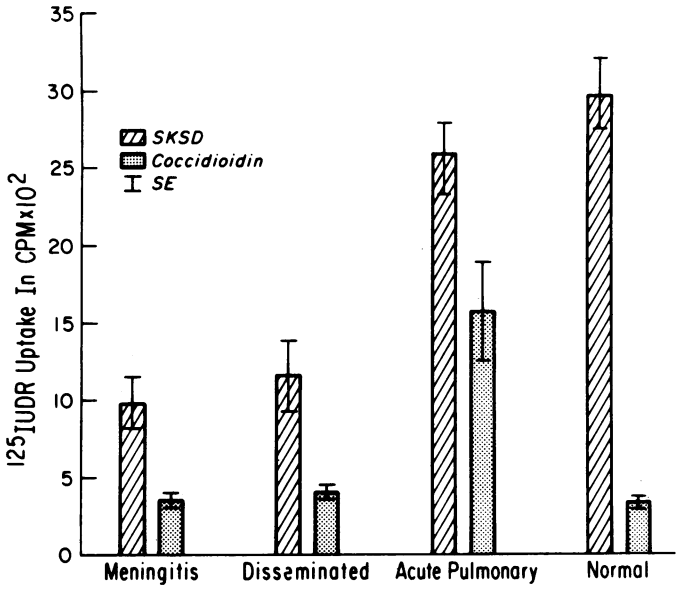

Figure 2.—Lymphocyte responses to antigens in patients with different forms of coccidioidomycosis. CPM = counts per minute, ^{125}IUDR = iododeoxyuridine I 125, SE = standard error.

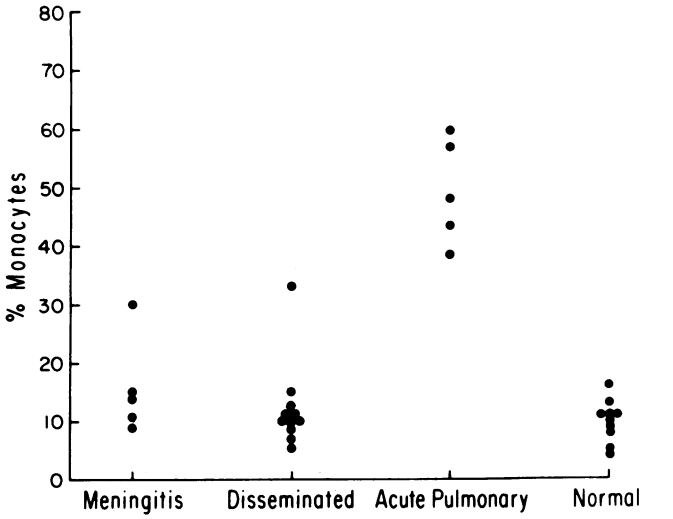

Figure 3.—Percentage of monocytes with two or more ingested coccidioidin-coated chicken erythrocytes in patients with coccidioidomycosis.

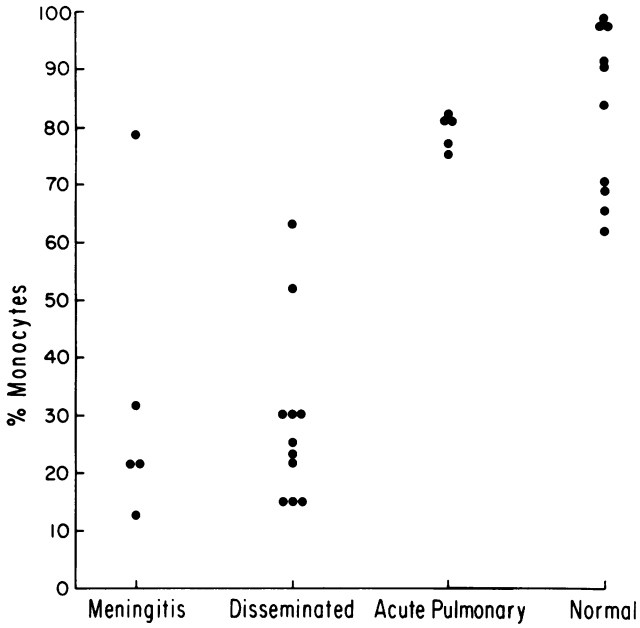

Figure 4.—Percentage of monocytes with two or more ingested human IgG-coated chicken erythrocytes in patients with coccidioidomycosis.

rates of erythrocyte ingestion as those observed in monocytes from patients with pulmonary diseases.

Discussion

These studies show that in the first months following infection, patients with meningeal and disseminated nonmeningeal coccidioidomycosis have impaired lymphocytic responses to mitogens and antigens and diminished monocytic capacity to ingest coccidioidin- and human IgG-coated chicken erythrocytes. The finding that lymphocytic transformation responses are reduced primarily in patients with extrapulmonary infection and that these reductions are most pronounced for coccidioidin accords with previous studies.[1,4,5] Because these patients were on amphotericin B or amphotericin B methyl ester at the time of study, it is possible that these agents may have contributed to the patients' reduced immunologic response. Because normal numbers of rosette-forming cells (T cells) were present in patients with meningitis, abnormality in T-cell function rather than number is probably responsible for the reduced immunologic response. The reduced immunologic response in patients with disseminated disease could be attributed to a reduced number of T cells as well as reduced functional capacity.

The reduced blastogenic lymphocyte response to coccidioidin did not always correlate with a lack of skin reactivity to the antigen. Four patients with extrapulmonary coccidioidomycosis had reduced lymphocyte blastogenic responsiveness and positive coccidioidin skin tests. This dissociation between skin and lymphocyte reactivity in patients with extrapulmonary disease has been noted by others and is unexplained.[3] Dermal reactivity occurred in the five patients with pulmonary infections, all of whom showed a blastogenic response to coccidioidin. This confirms previous reports that these two measurements of cell-mediated immunity usually agree in this form of the disease. Five of the eight patients with extrapulmonary infections and lack of delayed hypersensitivity response died compared with one death in the four patients who had dermal reactivity, suggesting that a positive delayed hypersensitivity test is associated with a more favorable clinical outcome.

The observation that monocytes from patients with extrapulmonary coccidioidomycosis have a defect in their ability to ingest coccidioidin- and IgG-coated chicken erythrocytes is a finding of significance. Mononuclear phagocytes are a major cellular defense against intracellular bacteria and fungi.[12-14] Studies in mice suggest that alveolar macrophages are important in the killing of *C immitis*.[15,16] Thus, the finding of an enhanced ingestive capacity for *C immitis*-coated chicken erythrocytes by monocytes from patients with pulmonary infections—but not those with more severe extrapulmonary disease—suggests that these monocytes would have similar differences in ingesting *C immitis* itself. That is, during the first months of infection patients whose monocytes are stimulated to ingest *C immitis* contain the infection within their lungs, whereas those whose monocytes fail to become activated are unable to halt the spread of infection. Because macrophages have an active role in both the afferent and efferent limbs of the immune response,[12,17] the phagocytic defect may either be responsible for or consequent to the T-cell impairment in blastogenic response to coccidioidin. Macrophages are involved in processing antigens for the T-cell response and an absence of this processing could account for the diminished T-cell response to coccidioidin. Alternatively, lymphokines from stimulated lymphocytes activate macrophages, increasing their phagocytic capacity, and a defect in this pathway may account for the impairment in ingestion. Regardless of the pathogenesis of the defect in monocyte function, the inability of monocytes from patients with extrapulmonary coccidioidomycosis to increase their ingestive capacity for coccidioidin-coated chicken erythrocytes is an important immunologic deficiency.

Our observations may aid clinicians faced with the dilemma of deciding the likelihood of extrapulmonary dissemination in a patient with a newly diagnosed coccidioidal infection. Detection of extrapulmonary lesions is critical in these patients because extrapulmonary infection warrants treatment with the highly toxic antifungal agent, amphotericin B, whereas localized pulmonary infection is usually self-limiting and need not be treated.[18] At present nuclear scanning with technetium Tc 99m methylene diphosphonate or gallium 67 is the most sensitive means for detecting extrapulmonary infection.[19,20] These procedures are recommended in patients in whom dissemination is suspected,[19,20] but because firm criteria for such suspicion do not exist, this important decision is often conjectural. Tests of immunologic function should help identify persons prone to dissemination, thereby improving the accuracy

of clinical judgments concerning the likelihood of extrapulmonary infection.

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Medical Practice Questions

EDITOR'S NOTE: *From time to time medical practice questions from organizations with a legitimate interest in the information are referred to the Scientific Board by the Quality Care Review Commission of the California Medical Association. The opinions offered are based on training, experience and literature reviewed by specialists. These opinions are, however, informational only and should not be interpreted as directives, instructions or policy statements.*

Assistant Surgeon for Laser Iridotomy

QUESTION:

Is it accepted medical practice to have an assistant surgeon present when a laser iridotomy is being done?

OPINION:

In the opinion of the advisory panel on ophthalmology, it is not necessary to have an assistant surgeon present when a laser iridotomy is done, except under special circumstances for selected patients such as those extremely ill.